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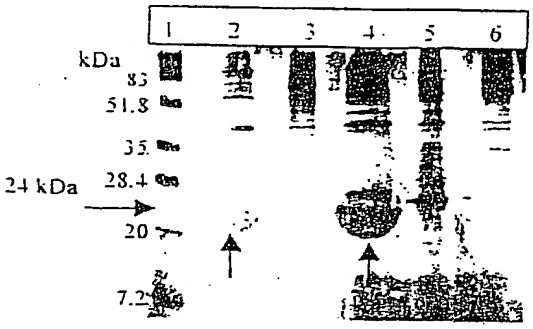
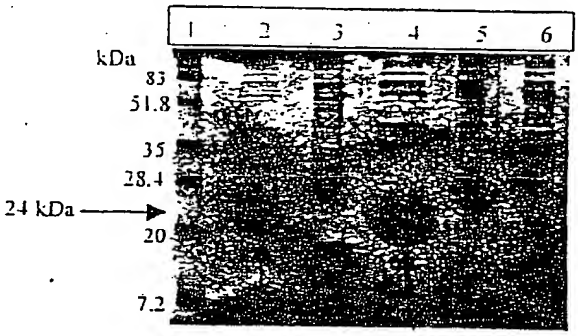
# Figure 1: Biopolymer-Proinsulin Fusion Protein Expression

A. Copper Stained




B. Coomassie R-250 Stained



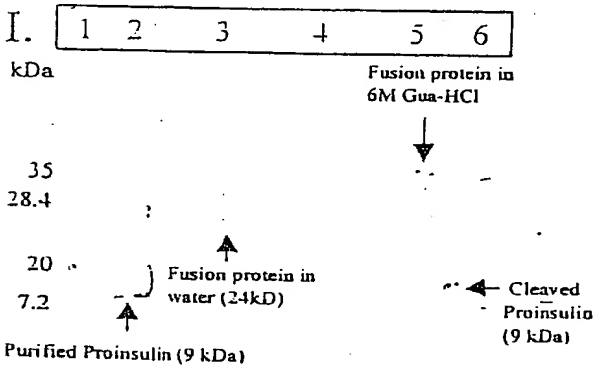

PAGE: 15% Glycine large gel

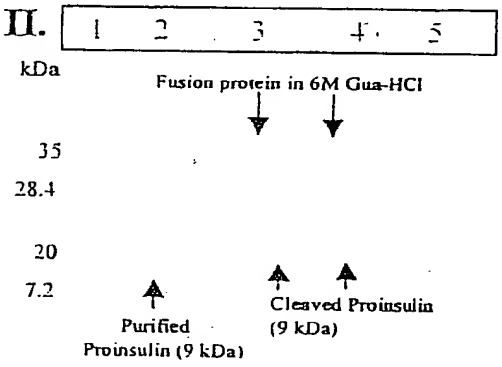
A. Copper Stained: Gel rinsed in water for 10 min, stained with 0.3M $CuCl_2$ for 5min, and rinsed in water for 3min.

B. Coomassie R-250 Stained: The same gel was first rinsed for 20min in water and then stained for 1hr, and destained overnight.

A. and B. Lanes. 1, Prestained Marker (BioRad) ; 2. Sonic extract of pSBL-OC-XaPris ; 3, reverse orientation of fusion protein of pSBL-OC- XaPris: 4. Sonic extract of pLD-OC-XaPris: 5, inverse orientation of pLD-OC- XaPris; 6. Sonic extract of *E. coli* strain XL-1 Blue containing no plasmid.

C. Western Blot of Biopolymer-Proinsulin Fusion Protein

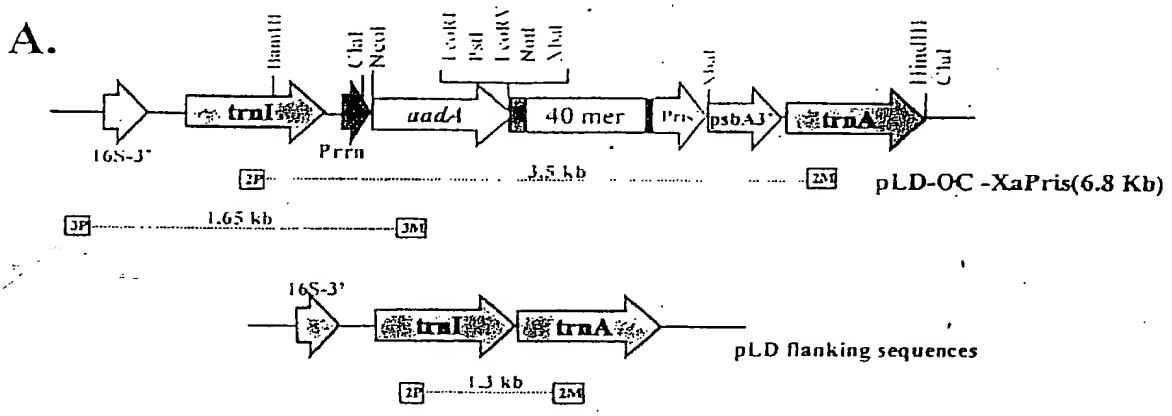


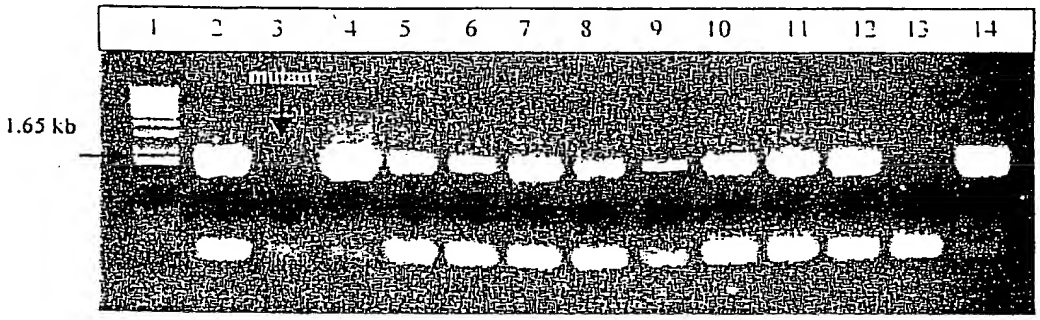

I. Lanes. 1, BioRad Prestained Marker; 2. 3ug of Purified Human Proinsulin; 3. 5ug of pSBL-OC-XaPris (sonication and purification of biopolymer twice); 4, Negative control, XL-1 Blue *E.coli*; 5. Sonic extract pSBL expressing cells (6M Guanidine Hydrochloride Phosphate Buffer, pH 7.0); 6, Sonic extract of XL-1 Blue *E.coli* with no pSBL.

II. Lanes. 1, BioRad Prestained Marker; 2. 5ug of Purified Human Proinsulin; 3, Sonic extract of pSBL-OC-XaPris expressing cells (6M Guanidine Hydrochloride Phosphate Buffer, pH 7.0); 4. Sonic extract of pLD-OC-XaPris expressing cells (Gua-HCl); 5. Sonic extract of XL-1 Blue *E.coli* with no plasmid.

# Figure 2: Confirmation of Chloroplast Integration by PCR of Polymer-Proinsulin Fusion Gene

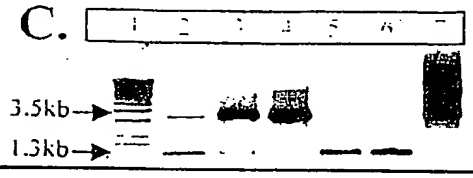


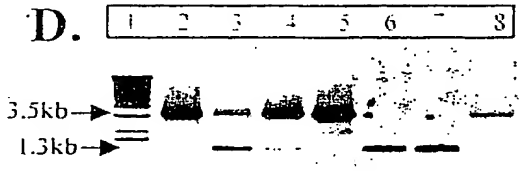

**B.** Confirmation of *aadA* integration into the chloroplast genome - Primers: **3P/3M**




A. Lanes, 1, 1 kb marker: 2. clone L19b (L=pLD-OC -XaPris) vector0; 3. clone L9 (mutant); 4. L1; 5, L8d: 6, L10a: 7. S30b (S=pSBL-OC -XaPris vector); 8. S20a; 9. S60: 10. S7a; 11. S28; 12. S41b: 13. Petit havana (not transgenic);14. Positive control (BADH gene present in chloroplasts from transgenic plants already confirmed)

Confirmation of integration of *aadA* and biopolymer-proinsulin fusion genes into the chloroplast genome - Primers: 2P/2M

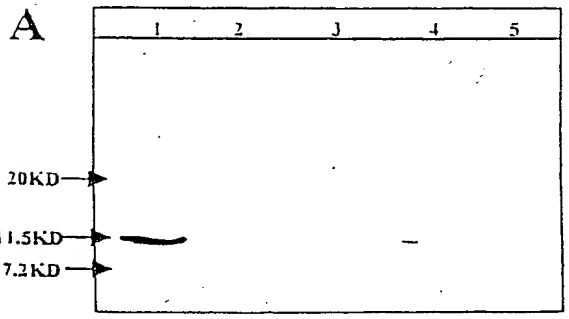



PCR of pLD clones: Lanes, 1, 1kb marker; 2, L17a; 3, L19b; 4. L8d; 5, L9; 6, Petit havana (not transgenic); 7. pLD vector as positive control

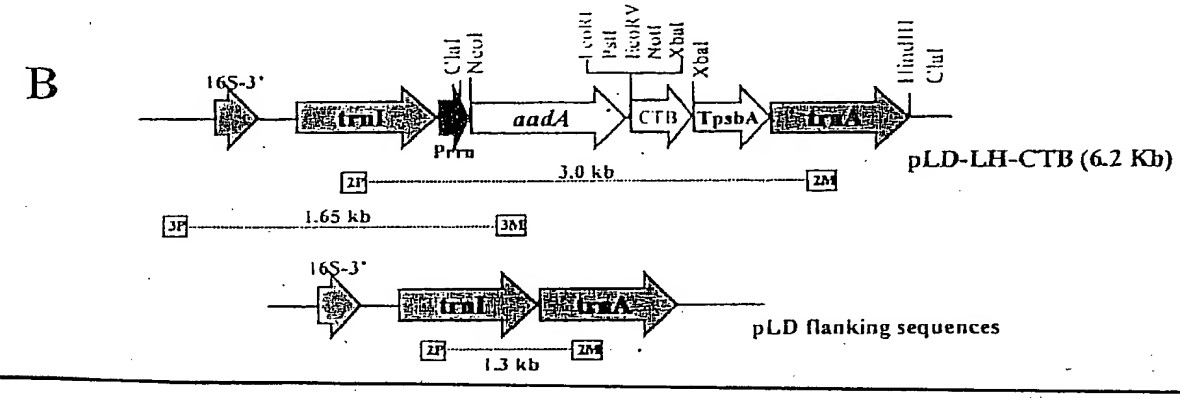
PCR of pSBL clones: Lanes, 1, 1kb marker; 2. S17a; 3, S30b; 4. S7a; 5, S41b;6. L9(mutant) 7. Petit havana (not transgenic); 8. pSBL vector as positive control

# Figure 3 : CTB Gene Expression and Chloroplast Integration

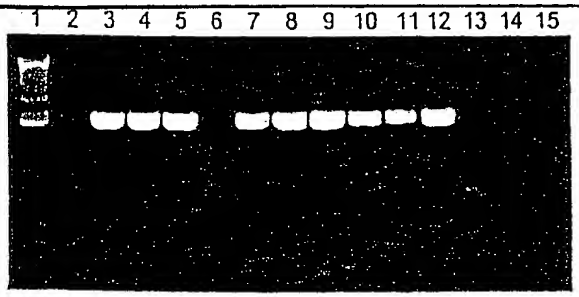



**Western Blot analysis of CTB expression in *E.coli* (15% PAGE):**

Lane 1: Purified bacterial CTB (0.5µg) ; 2 & 4: Transformed E.coli culture-24 h and 48 h resply. ; 3 & 5 : Untransformed E.coli culture- 24 h and 48 h resply.



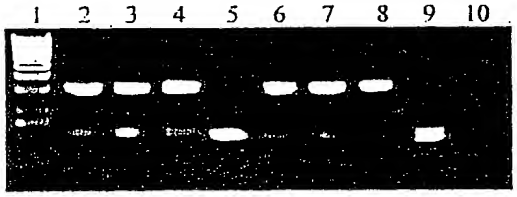

**C.** PCR confirmation of *aadA* gene integration into chloroplast genome -3P/3M primers




**PCR of clones of 1st. round of selection :**

Lane 1:1 Kb marker ; 2 - 12 : Plant total DNA from spec.r clones 1-11 (Note: Lanes 2 & 6 are mutants); 13: Untransformed plant; 14: pLD-LH-CTB vector ; 15: No DNA template

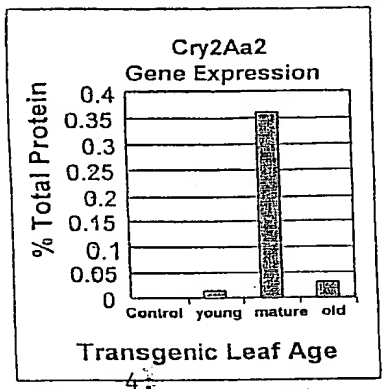
**D.** PCR confirmation of integration of *aadA* and CTB gene into chloroplast genome - 2P/2M primers




**PCR of clones of 2nd. round of selection :**

Lane 1: 1 Kb marker; 2 - 7 : Plant total DNA from spec.r clones 1- 6 (Note: Lane 5 is a mutant); 8: pLD-LH-CTB vector; 9: Untransformed plant ; 10 : No DNA template.

• Expression of bacterial operon in transgenic chloroplasts.



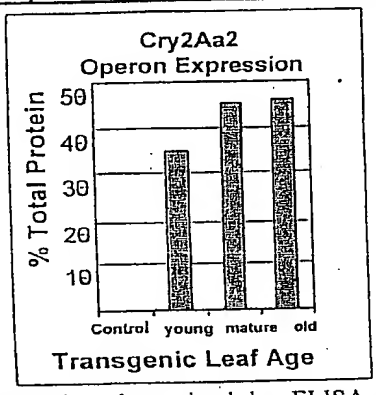




Figure 1: Cry2A protein concentration determined by ELISA in transgenic leaves. Note 100-fold increase in protein accumulation in the presence of the putative chaperonin, ORF2.

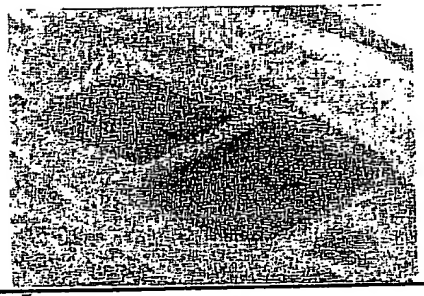


Figure 2 :Inmunogold labeled electron microscopy of mature transgenic leaf. Cry2Aa2 crystals in a transgenic chloroplast expressing the cry2A operon.

• Expression of a small (22aa) peptide in transgenic chloroplasts.

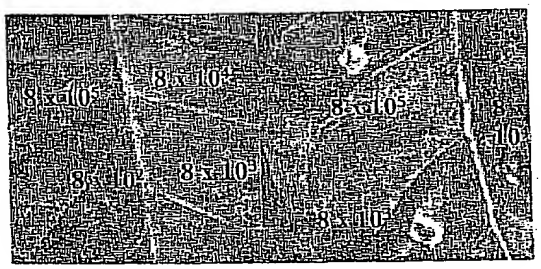



Figure 3: Leaves were infected with 10 µl of $8x10^5$, $8x10^4$, $8x10^3$ and $8x10^2$ cells of *P. syringae*. Photos were taken 5 days after inoculation. 1-2 µg of antimicrobial peptide (AMP) is required to kill 1000 bacterial cells. Local concentration at the site of infection is estimated to be 200-800µg AMP.

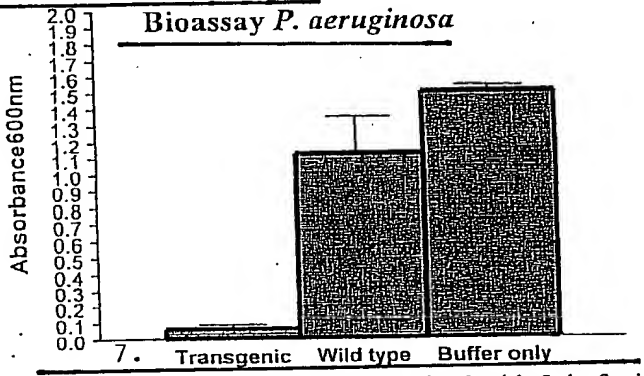
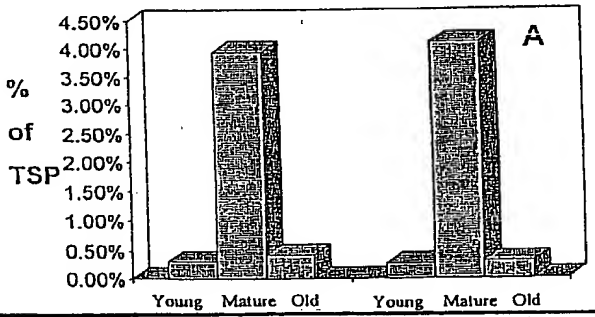



Figure 4. Total plant protein was mixed with 5µl of mid-log phase bacteria from overnight culture, incubated for 2 hours at 25°C at 125rpm and grown in LB broth overnight. Based on minimum inhibitory concentration of 1-2 µg AMP/1000 bacterial cells, the expression level was calculated to be 21.5-43% of the total soluble protein.

• Expression of Oligomeric form (disulfide bonded) CTB in transgenic chloroplasts.



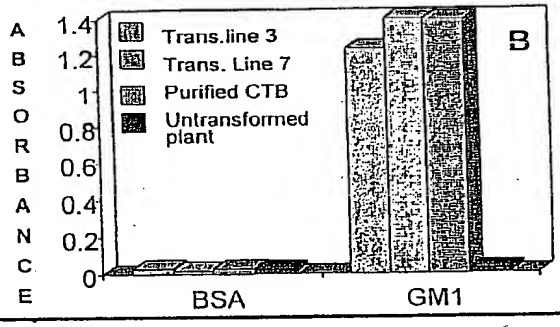




Figure 5: A) CTB ELISA quatification is shown as a percentage of the total soluble plant protein. Total soluble plant protein from young, mature and old leaves of transgenic lines 3 and 7 was quantified. B) CTB-GM1 Ganglioside binding ELISA assays: Plates coated first with GM1 gangliosides and BSA were plated with total soluble plant protein from lines 3 and 7, untransformed plant total soluble protein and purified bacterial CTB. The absorbance or the GM1 ganglioside-CTB antibody complex was measured.

## • Expression of CTB oligomers.

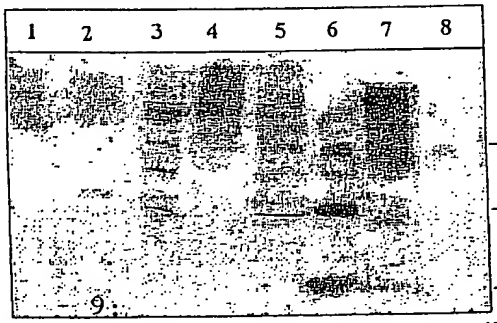



Figure 6: 12% reducing PAGE. Chemiluminescent detection with rabbit anti-cholera serum (1°) and AP labeled mouse anti-rabbit IgG (2°) antibodies. Untransformed, boiled(1) and unboiled (2); Transformed, boiled (3&5) and unboiled (4);Purified CTB boiled (6)and unboiled (7); Marker (8).

## • Expression & assembly of disulfide bonded Guy's 13 monoclonal antibody.

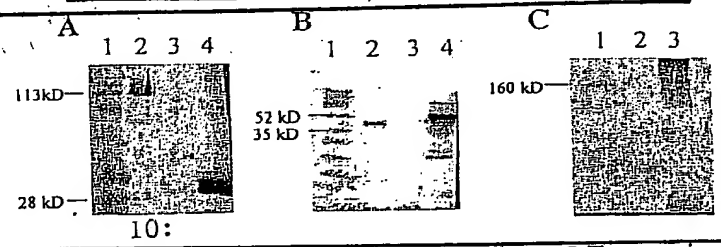



Figure7: A, B) reducing gels. 1:markers, 2:Transgenic extract showing expression of light (A) and heavy chain (B) in chloroplasts, 3: Untransformed, 4: Human IgA, C) non-reducing gel. 1.Transgenic extract showing assembly, 2: Untransformed, 3: Human IgA. Blots A & C were detected with AP conjugated goat anti-human kappa antibody. Blot B was detected with AP conjugated goat anti-human IgA antibody.

## •Marker-free chloroplast transgenic plants.

| Selectable marker | Plate No. | Total no. of leaf discs | No of responding leaf discs | Total no of shoots/ plate |
|---|---|---|---|---|
| BADH | 1 | 3 | 3 | 43 |
| | 2 | 6 | 4 | 23 |
| | 3 | 11 | 9 | 33 |
| | 4 | 7 | 6 | 19 |
| | 5 | 6 | 4 | 16 |
| | 6 | 9 | 7 | 18 |
| Spectinomycin | 1 | 5 | 0 | 0 |
| | 2 | 5 | 0 | 0 |
| | 3 | 5 | 3 | 3 |
| | 4 | 5 | 2 | 2 |
| | 5 | 5 | 0 | 0 |
| | 6 | 5 | 1 | 1 |
| | 7 | 5 | 1 | 2 |
| | 8 | 5 | 1 | 2 |
| | 9 | 5 | 0 | 0 |
| | 10 | 5 | 0 | 0 |
| Control | | 5 | 0 | 0 |

Table 1: Comparison of Spectinomycin and Betaine aldyhyde as the selectable marker for the first round of selection.

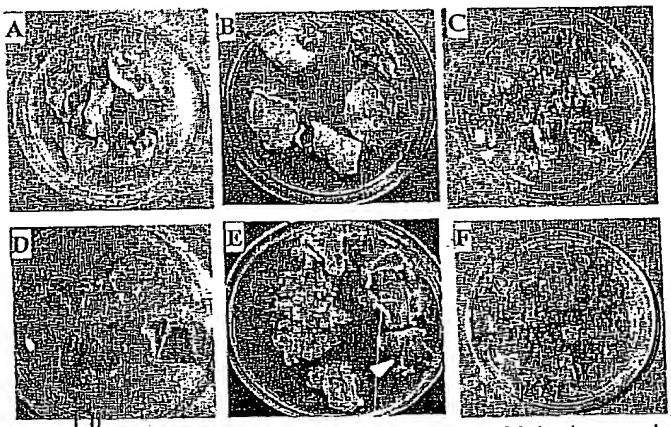


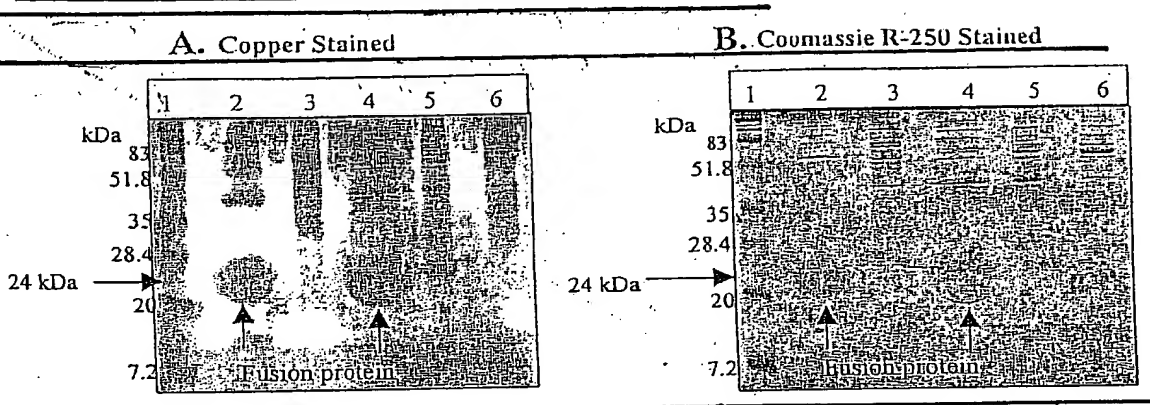

Figure 8: Comparison of betaine aldehyde and spectinomycin selection. A. *N. tabacum* Petit Havana control in RMOP medium containing spectinomycin after 45 days. B. Bombarded leaf discs selected on spectinomycin in RMOP medium after 45 days. C. Spectinomycin resistant clones cultured again (second round) to obtain homoplasmy. D. Petit Havana control in RMOP medium containing Betaine Aldehyde after 12 days of culture. E. Bombarded leaf discs selected on Betaine Aldehyde in RMOP medium after 12 days of culture; arrow indicates unbombarded leaf disc as control. Note that 23 shoots are formed on a disc selected on betaine aldehyde against 1-2 shoots per disc on spectinomycin. F. Betaine aldehyde resistant clones cultured again (second round) to obtain homoplasmy.

## • Codon composition and expression levels.

| Open reading Frame | % TSP | % A+T | % psbA | %cp tRNA |
|---|---|---|---|---|
| Plastid miniproinsulin | ? | 66 | 100 | 62 |
| CTB | 4.1 | 66 | 47 | 34 |
| Cry2A operon | 47 | 65 | 37 | 37 |
| Plastid proinsulin | ? | 64 | 100 | 49 |
| Antimicrobial peptide | 21 | 63 | 35 | 35 |
| Guy's light chain | <1% | 49 | 31 | 44 |
| Optimized biopolymer | ? | 47 | 100 | 40 |
| Guy's heavy chain | <1% | 40 | 25 | 44 |
| Human proinsulin | ? | 38 | 26 | 44 |

Table 2 (Left): Black indicates genes with unmodified native codon composition and their expression levels observed in transgenic chloroplasts, ranked by AT% in ascending order. Red indicates genes to be investigated. Kusnadi et al. (1997) suggest that a minimum of 1% TSP is adequate for commercial feasability. See section d) for details of AT content, %psbA optimal codons and % of codons that match the cp tRNA pool. TSP: % total soluble protein

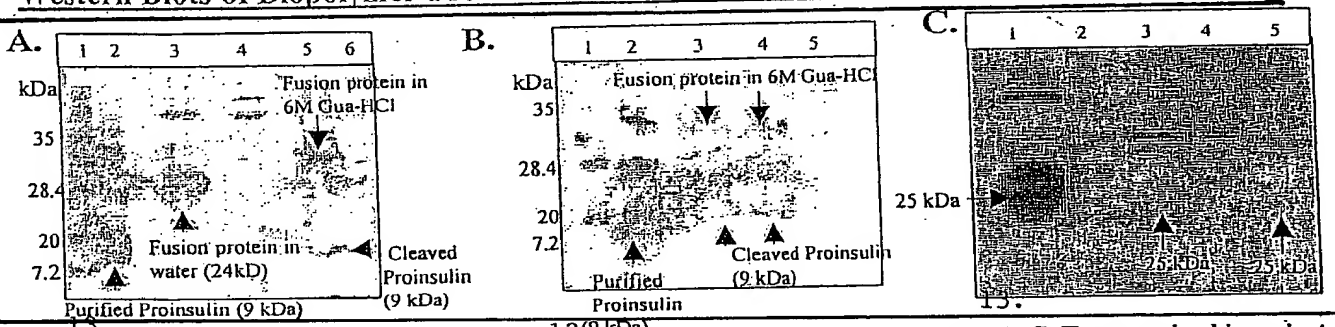
## Biopolymer-Proinsulin Fusion Protein Expression




12.

Fig 9, A and B Lanes: 1, Prestained Marker (BioRad) ; 2, Sonic extracts of pSBL-OC-XaPris ; 3, reverse orientation of insert in pSBL-OC- XaPris; 4, pLD-OC-XaPris; 5, reverse orientation of pLD-OC- XaPris; 6, *E. coli* XL-1 Blue cells with no plasmid .

## Western Blots of Biopolymer-Proinsulin Fusion Protein After Single Step purification




15.

**Fig 10: A. E. coli expression and cleavage** Lanes: 1, BioRad Prestained Marker; 2, 3ug of Purified Human Proinsulin; 3, 5ug of pSBL-OC-XaPris; 4, Negative control, reverse orientation; 5, pSBL expressing cells (6M Guanidine Hydrochloride Phosphate Buffer, ph7.0); 6, XL-1 Blue *E.coli* with no pSBL.

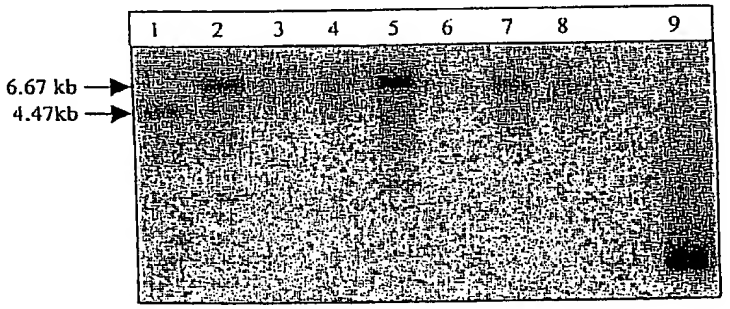
13

**Fig 10: B. E. coli expression and cleavage** Lanes: 1, BioRad Prestained Marker; 2, 5ug of Purified Human Proinsulin; 3, pSBL-OC-XaPris (6M Guanidine Hydrochloride Phosphate Buffer, ph7.0); 4, pLD- OC-XaPris; 5, XL-1 Blue *E.coli* with no plasmid.

15.

**Fig 10: C. Transgenic chloroplast expression** Lanes: 1, Purified *E. coli* protein from pLD-OC-XaPris expression; 2, negative control (Petit Havana); 3-5, Chloroplast transgenic lines. Note dimer, tetramer and hexamer aggregates of polymer-insulin fusion protein

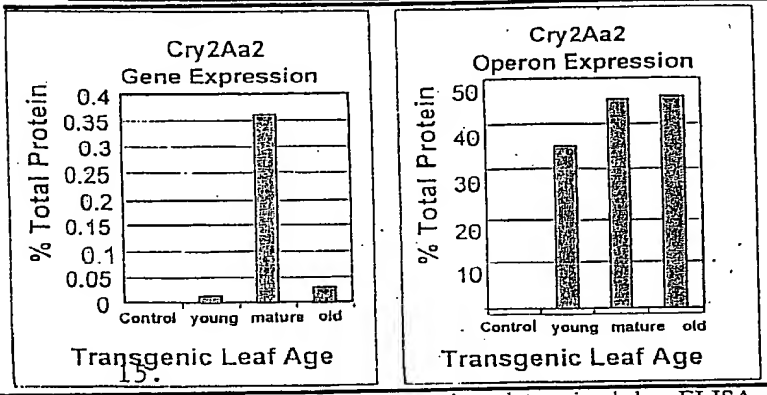
## Confirmation of chloroplast integration and homoplasmy/heteroplasmy by Southern Blot Analysis




14.

Fig 11. Biopolymer-proinsulin fusion gene integration into the chloroplast genome confirmed by Southern blot analysis. Lanes: 1, Petit Havana (negative control); 2-5, pLD-OC-XaPris clones $T_0$; 6-8, pSBL-OC-XaPris clones $T_0$; 9, probe(positive control). Homoplasmy is seen in most transgenic lines while a few transgenic lines show heteroplasmy.

• Expression of bacterial operon in transgenic chloroplasts.



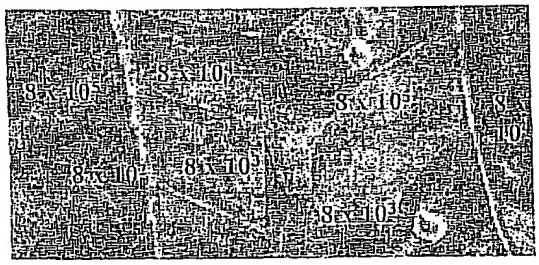

15.

Figure 1: Cry2A protein concentration determined by ELISA in transgenic leaves. Note 100-fold increase in protein accumulation in the presence of the putative chaperonin, ORF2.

16.

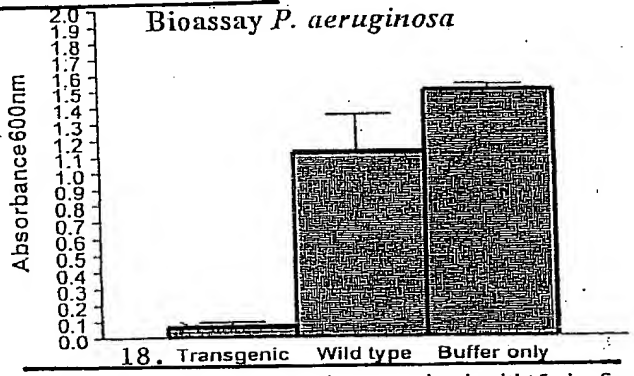
Figure 2 :Inmunogold labeled electron microscopy of mature transgenic leaf. Cry2Aa2 crystals in a transgenic chloroplast expressing the cry2A operon.

• Expression of a small (22aa) peptide in transgenic chloroplasts.




17.

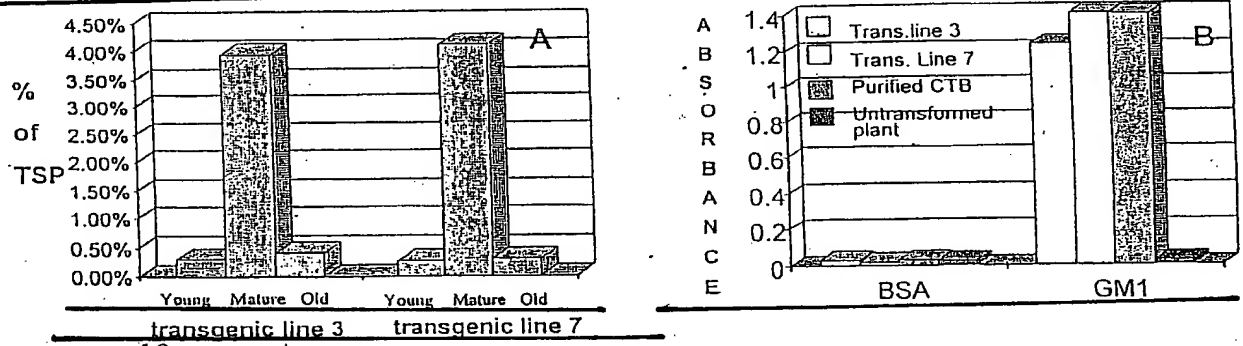
Figure 3 : Leaves were infected with 10 µl of $8x10^5$, $8x10^4$, $8x10^3$ and $8x10^2$ cells of *P. syringae*. Photos were taken 5 days after inoculation. 1-2 µg of antimicrobial peptide (AMP) is required to kill 1000 bacterial cells. Local concentration at the site of infection is estimated to be 200-800µg AMP.




18.

Figure 4: Total plant protein was mixed with 5µl of mid-log phase bacteria from overnight culture, incubated for 2 hours at 25°C at 125rpm and grown in LB broth overnight. Based on minimum inhibitory concentration of 1-2 µg AMP/1000 bacterial cells, the expression level was calculated to be 21.5-43% of the total soluble protein.

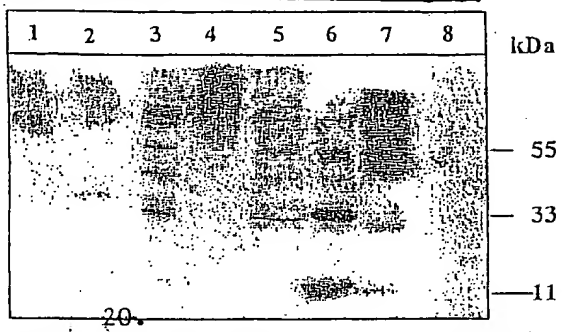
• Expression of Oligomeric form (disulfide bonded) CTB in transgenic chloroplasts.




19.

Figure 5: A) CTB ELISA quatification is shown as a percentage of the total soluble plant protein. Total soluble plant protein from young, mature and old leaves of transgenic lines 3 and 7 was quantified. B) CTB-GM1 Ganglioside binding ELISA assays: Plates coated first with GM1 gangliosides and BSA were plated with total soluble plant protein from lines 3 and 7, untransformed plant total soluble protein and purified bacterial CTB. The absorbance or the GM1 ganglioside-CTB antibody complex was measured.

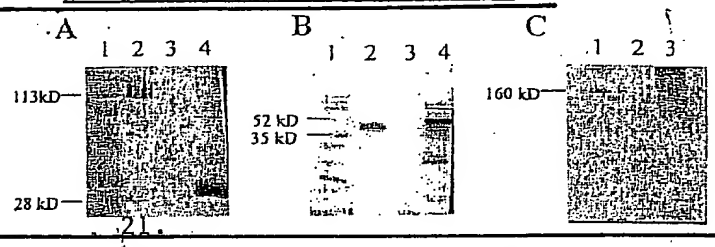
• Expression of CTB oligomers.




20.

Figure 6: 12% reducing PAGE. Chemiluminescent detection with rabbit anti-cholera serum ($1^0$) and AP labeled mouse anti-rabbit IgG ($2^0$) antibodies. Untransformed, boiled(1) and unboiled (2); Transformed, boiled (3&5) and unboiled (4);Purified CTB boiled (6)and unboiled (7); Marker (8).

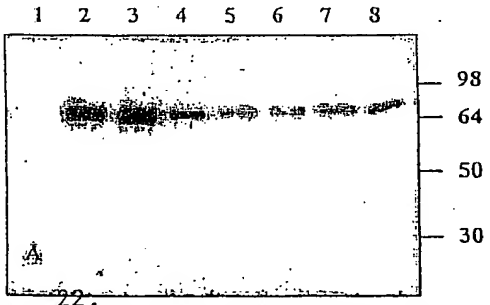
• Expression & assembly of disulfide bonded Guy's 13 monoclonal antibody .




21.

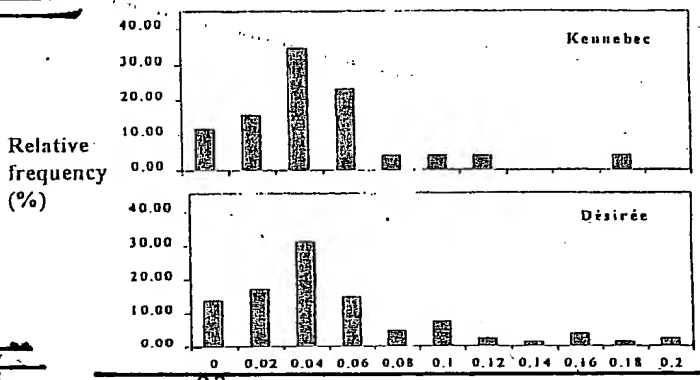
Figure 7: A, B) reducing gels. 1:markers. 2:Transgenic extract showing expression of light (A) and heavy chain (B) in chloroplasts, 3: Untransformed, 4: Human IgA: C) non-reducing gel. 1.Transgenic extract showing assembly, 2: Untransformed, 3: Human IgA. Blots A & C were detected with AP conjugated goat anti-human kappa antibody. Blot B was detected with AP conjugated goat anti-human IgA antibody.

• HSA Nuclear transformation of potato plants .




22.

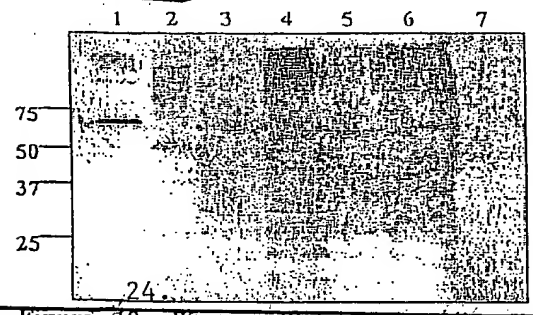
Figure 8: *Western Blot* of transgenic potato tubers, cv Desiree. 30 µg of tuber protein was loaded per lane and probed with anti-HSA antibody. 1: wild type; 2: 40 ng of pure HSA; 3-8:diferent trangenic lines, showing different levels of expression.




23.

Figure 9: Frecuency histogram including percentage Kennebec and Désirée transgenic plants expressing different HSA levels. Results are shown as the percentages of transgenic plants (vertical axis) that express a specific level of HSA of the total soluble protein (horizontal axis).

• Expression of HSA by chloroplast vectors in *E. coli*.




24.

Figure 10: *Western Blot* of *E. coli* protein extracts. 1: 50 ng pure HSA; 2: molecular weigh marker; 3: pLD-HSA (control without RBS); 4: PLD- 5'UTR-HSA; 5: pLD-RBS-HSA; 6: pLD-ORF1+2-HSA; 7: *E. coli* without pLD vector.

• Codon composition and expression levels.

| Open reading Frame | % TSP | % A+T | % psbA | %cp tRNA |
|---|---|---|---|---|
| CTB | 4 | 66 | 47 | 34 |
| Cry2A operon | 47 | 65 | 37 | 37 |
| Antimicrobial peptide | 21-43 | 63 | 35 | 35 |
| HSA | ? | 57 | 57 | 47 |
| Interferon alpha | ? | 54 | 31 | 40 |
| RUBISCOssTP | ? | 50 | 32 | 42 |
| Guy's light chain | <1% | 49 | 31 | 44 |
| IGF-I | ? | 41 | 20 | 30 |
| Guy's heavy chain | <1% | 40 | 25 | 44 |

Table 1: Unmodified native codon composition and expression levels observed in transgenic chloroplasts. See section d) for details of AT content, %psbA optimal codons and % of codons that match the cp tRNA pool. TSP: % total soluble protein

• Expression of HSA via chloroplast genome in tobacco.

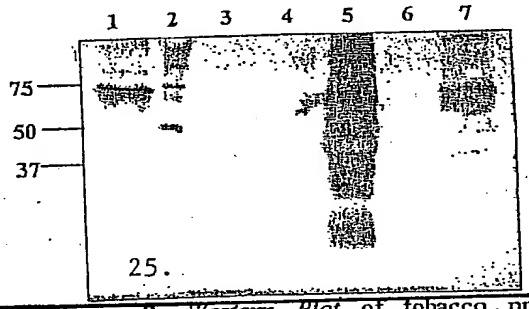
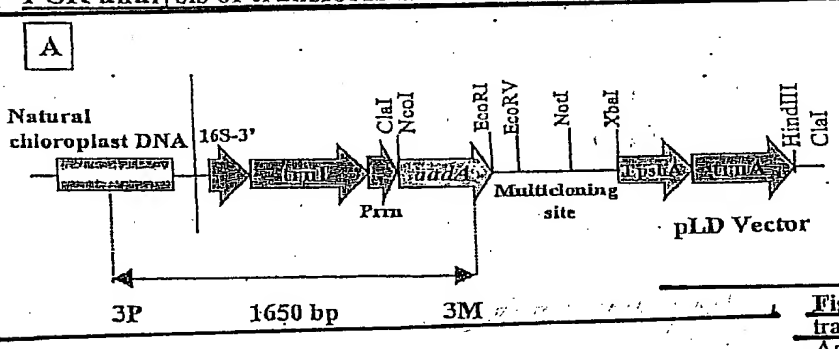



Figura 9: *Western Blot* of tobacco protein extracts. 1: 40 ng pure HSA; 2: molecular weight marker; 3 and 4: wild type plant extracts; 5: extracts form plants transformed with PLD-5'UTR-HSA; 6: pLD-RBS-HSA; 7: pLD-ORF1+2-HSA. 30 micrograms of plant protein were loaded per well.

•PCR analysis of transformants to determine integration of HSA gene into the chloroplast genome.



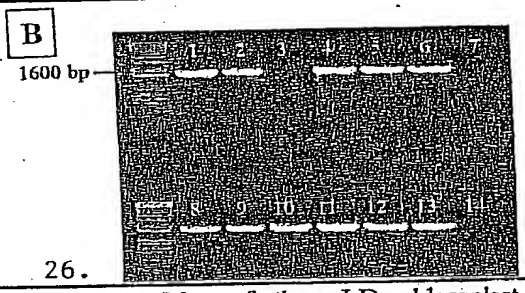
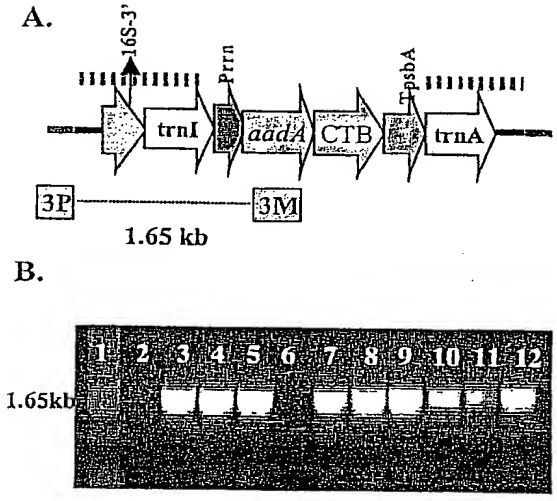
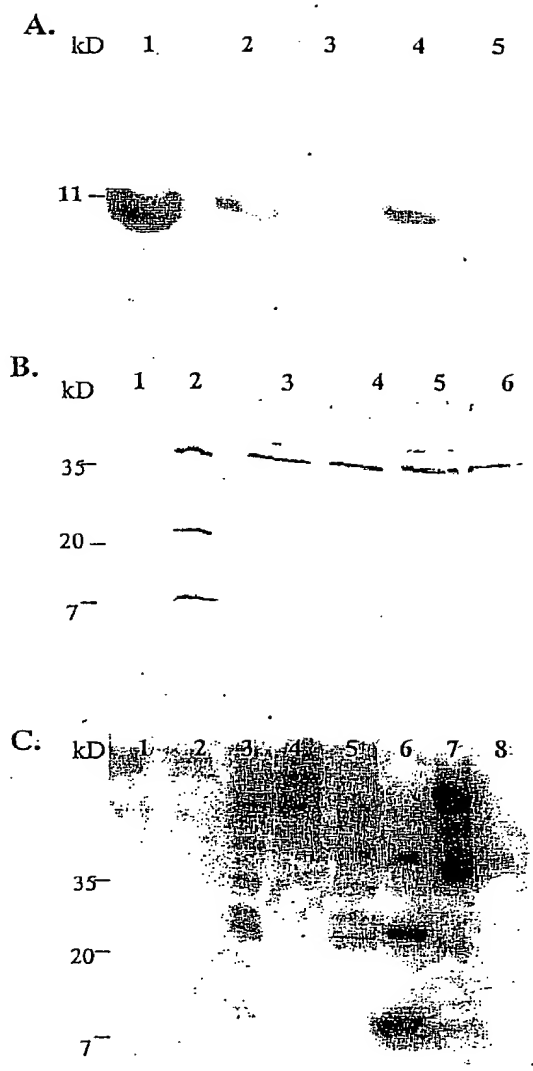




Figura 10: A) Map of the pLD chloroplast transformation vector and primer landing sites. B) Agarose gel containing PCR products using total plant DNA as template from plants transformed with: 1,2,3: pLD-RBS-HSA; 4,5,6: pLD-5'UTR-HSA; 8,9,10: pLD-ORF1+2-HSA; 11,12,13: pLD-ORF1+2-5'UTR-HSA; 7,14: negative controls (from untransformed plants); 3: mutant.


A.
16S-3'
Prrn
TpsbA
trnI
aadA
CTB
trnA
3P
3M
1.65 kb
B.
1 2 3 4 5 6 7 8 9 10 11 12
1.65kb

27.

**Figure 1**




28.

**Figure 2**

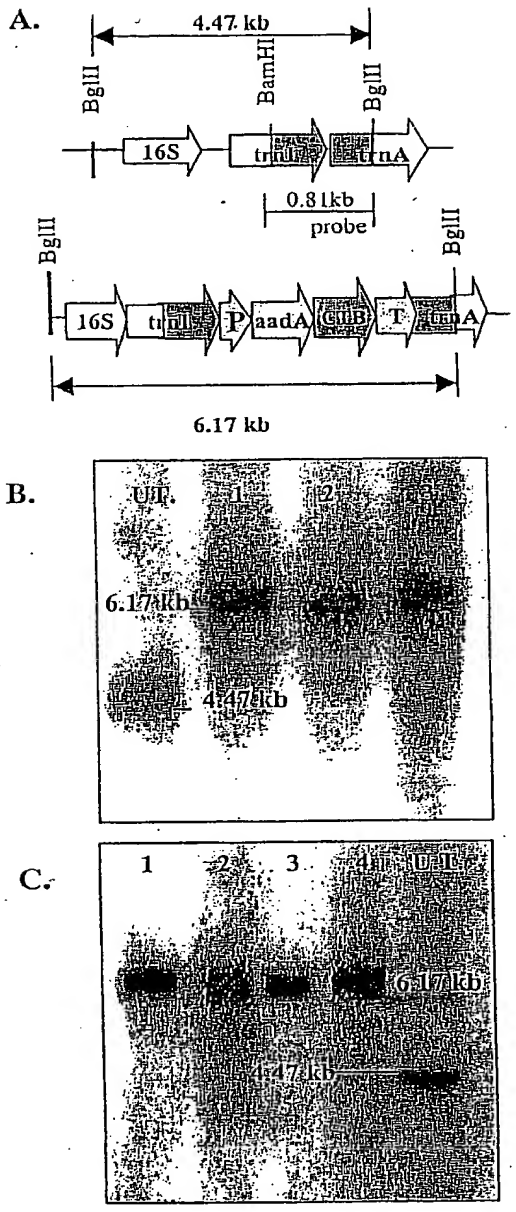
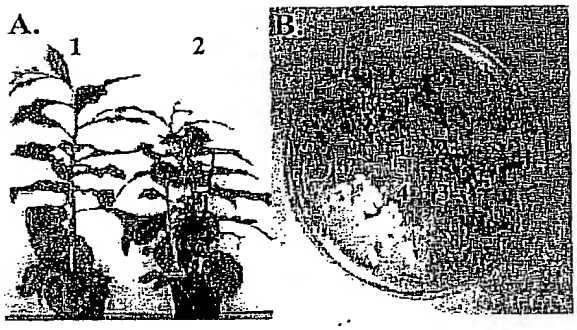

A.
4.47 kb
BglII
BamHI
BglII
16S
trnI
trnA
0.8 kb
probe
BglII
BglII
16S
trnI
P
aadA
ClpB
T
trnA
6.17 kb
B.
UT.
1
2
6.17kb
4.47kb
C.
1
2
3
4
UT.
6.17kb
4.47kb



**Figure 3**




30.

**Figure 4**

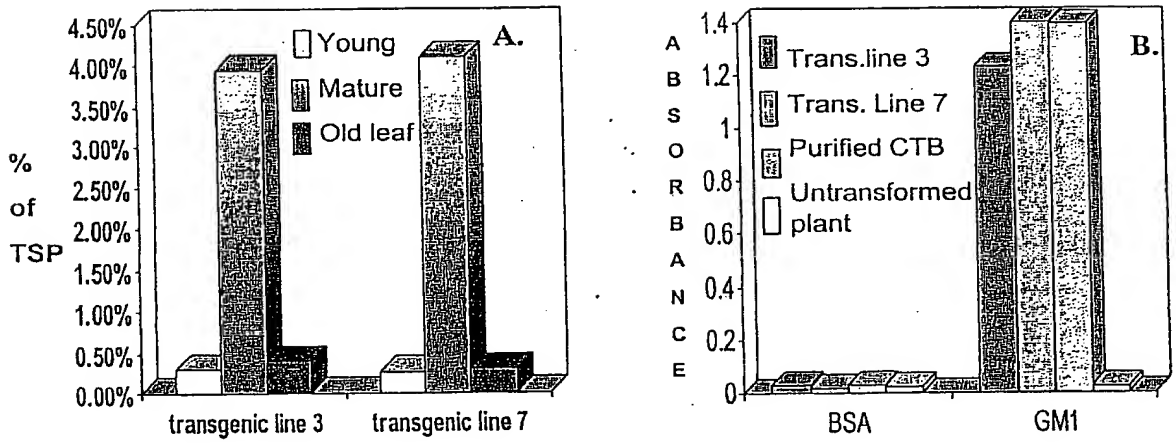
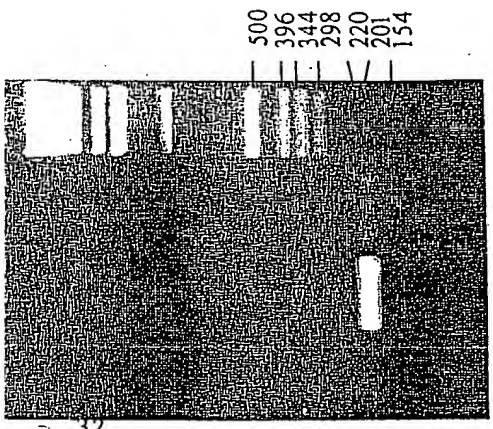

A.
% of TSP
4.50%
4.00%
3.50%
3.00%
2.50%
2.00%
1.50%
1.00%
0.50%
0.00%
Young
Mature
Old leaf
transgenic line 3
transgenic line 7
B.
ABSORBANCE
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Trans.line 3
Trans. Line 7
Purified CTB
Untransformed plant
BSA
GM1

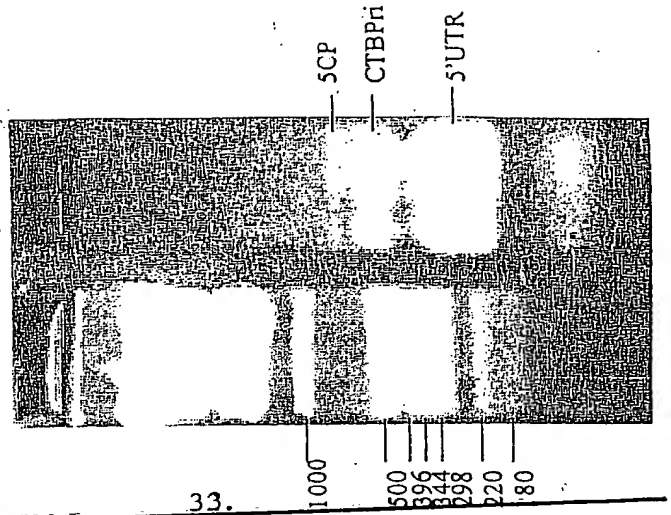


**Figure 5**




32.

re 1 (above): cloning of the PsbA 5' untranslated (5'UTR) from the chlorplast genome




33.

**Figure 2** (above): SOEing of the 5'UTR to the CTB- human proinsulin sequence. **5CP** is the PSbA 5'UTR and the Cholera Toxin B subunit (CTB) human proinsulin fusion

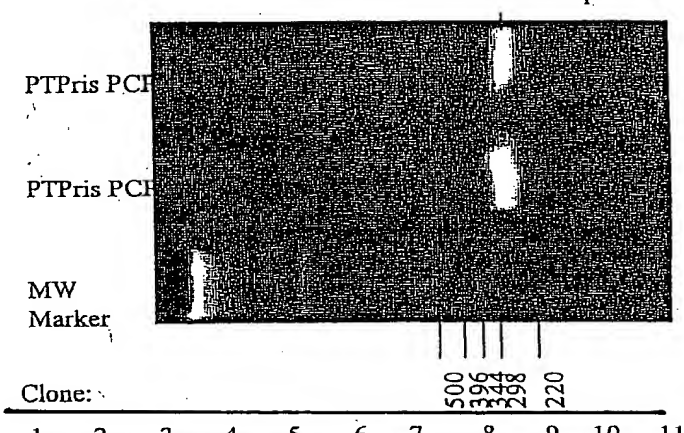
34

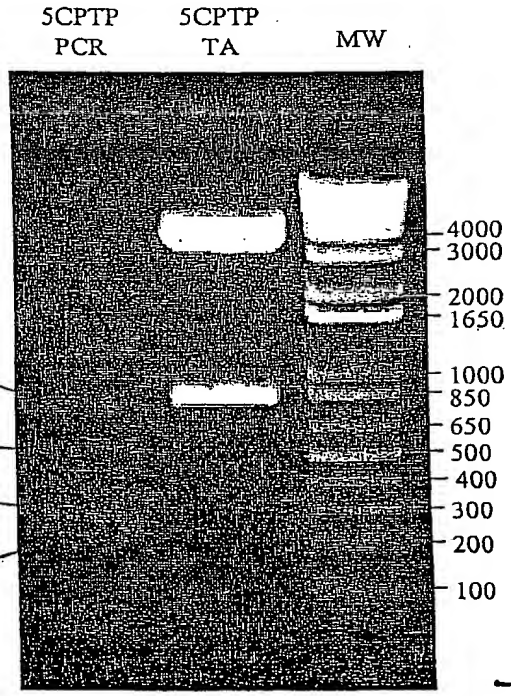
re 3 (below): a comparisison of the DNA sequences of native proinsulin (top) and plastid modified proinsulin (bottom)

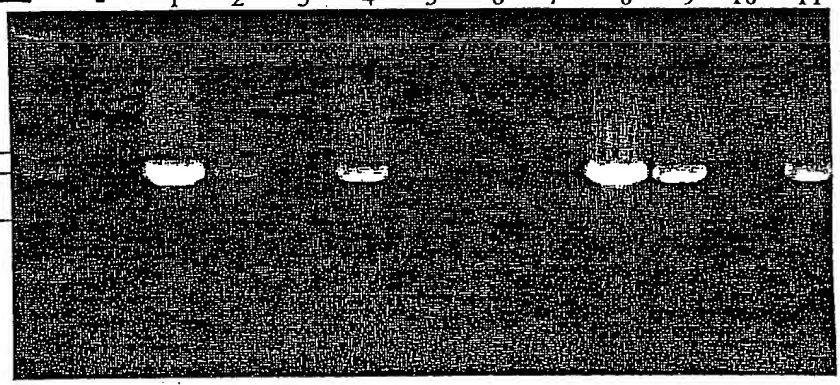
```
tgaaccaacacctgtgcggctcacacctggtggaagctctctacctagtgtgcggg
| ||||||||| | || || || ||||| || |||||| | ||| |||| || ||
taaaccaacacttatgtggttctcacctagtagaagctttatacttagtatgtggt

gaggcttcttctacacacccaagacccgccgggaggcagaggacctgcaggtgggg
| || ||||||||||| || || || || || || || || || || | || || ||
gtggtttcttctacactcctaaaactcgtcgtgaagctgaagatttacaagtaggt

tggagctgggcgggggccctggtgcaggcagcctgcagcccttggccctggagggg
| || | || || || |||||||| || | || || || || | || ||
tagaattaggtggtggtcctggtgctggttctttacaacctttagctttagaaggt

tgcagaagcgtggcattgtggaacaatgctgtaccagcatctgctccctctaccag
| || || ||||| ||||| |||||||| ||||| || || || | |||||
tacaaaaacgtggtattgtagaacaatgttgtacttctatttgttctttataccaa

agaactactgcaacta   Native Human Proinsulin
| |||||||| |||||
aaaactactgtaacta   Chloroplast Modified Proinsulin
```

35

**Figure 4** (below): Recursive PCR to synthesize the chloroplast modified proinsulin (Ptpris)




37

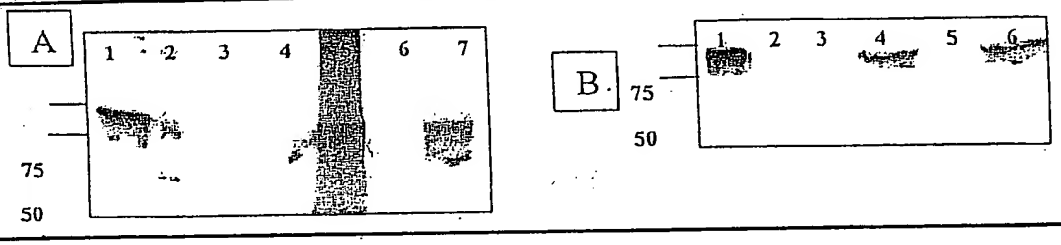
**Figure 6** (above): PCR products to confirm construct integration into the chloroplast genome using two primers, 3P and 3M. 3P anneals to the native chloroplast genome and 3M anneals to the introduced spectinomycin resistance gene, *aadA*, creating a 1600 bp product only in transgenic clones

36.

**Figure 5** (left): SOEing of the 5'UTR, CTB, and plastid modified proinsulin, which results in the fusion of all three sequences denoted as **5CPTP**. The second lane show this

promoter. IFNα5 gene was cloned into the pLD using both sequences and bombarded into tobacco leaves. Shoots appeared after 5 weeks and the second round of selection is in progress.

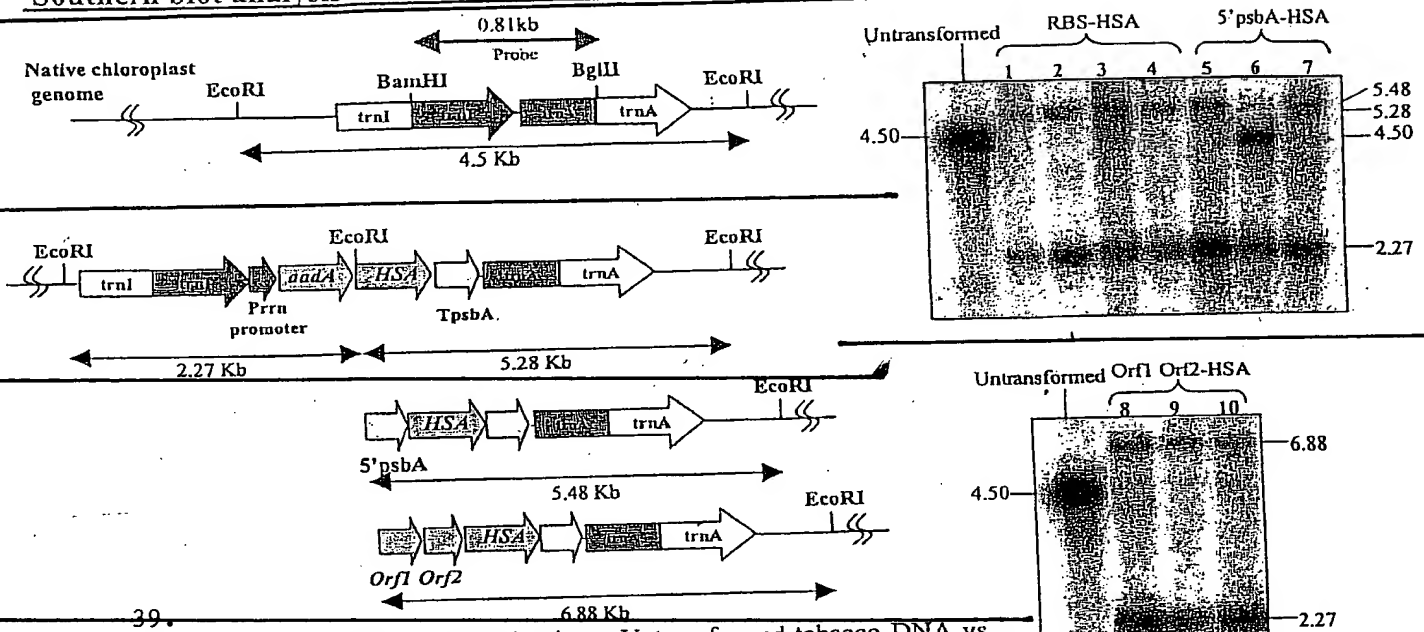
· **Expression of HSA via the chloroplast genome in tobacco.**




38.

Figure 1: *Western Blot* of tobacco protein extracts. A) 1: 40 ng pure HSA; 2: molecular weight marker; 3,4,6: untransformed plant extracts; 5: extract from plants transformed with: PLD- 5'UTR-HSA; 7: pLD-Orf1Orf2-HSA. B) 1: 40 ng pure HSA; 2: molecular weight marker; 3,5: untransformed plant extracts; 4: extract from plants transformed with: PLD- RBS-HSA; 6: pLD-Orf1Orf2-HSA. 10 micrograms of plant protein were loaded in each well.

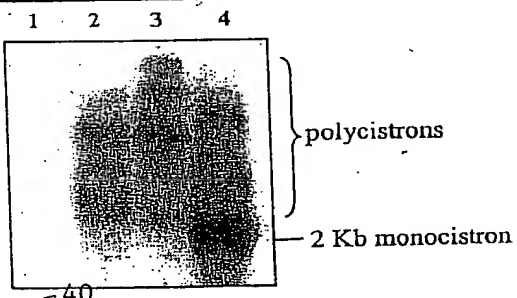
• Southern blot analysis of HSA transgenic tobacco plants.




39.

Figure 2: *Southern Blot* of HSA transgenic plants. Untransformed tobacco DNA vs. transgenic tobacco DNA digested with EcoRI. 1,2,3,4: DNA from plants transformed with pLD-RBS-HSA; 5,6,7: pLD-5'psbA-HSA; 8,9,10: pLD-Orf1-Orf2-HSA. Note homoplasmy in all the clones except number 6.

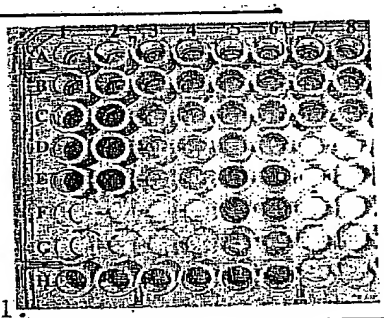
• Northern blot analysis of HSA transgenic tobacco plants.




40

Figure 3: *Northern Blot* of HSA transgenic plants using HSA probe (1.8 kb). 1: untransformed tobacco RNA. 2: RNA from plants transformed with: pLD-RBS-HSA; 3: pLD-Orf1-Orf2-HSA; 4: pLD-5'psbA-HSA. Note different sizes of transcripts and the presence of monocistrons in number 4.

• ELISA analysis of HSA transgenic tobacco plants.



41.

Figure 4: *ELISA* of HSA transgenic plants. A-E/1-2: HSA standards; F/1-2: Blank; G/1-2: Untransformed Petit Havana protein extracts; D-E/3-4: proteins from plants transformed with pLD-Orf1Orf2-HSA; F-G/3-4 and D-H/7-8: pLD-RBS-HSA; Rest of the wells contain extracts from different clones transformed with pLD-5'psbA-HSA.

• IGF-I optimized sequence and PCR product after synthesis of the new gene.

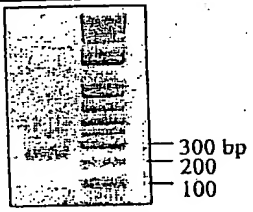
A

```
ggaccggagacgctctgcggggctgagctggtggatgctcttcagttcgtg
tgtggagacaggggcttttatttcaacaagcccacagggtatggctccagc
agtcggagggcgcctcagacaggcatcgtggatgagtgctgcttccggagc
tgtgatctaaggaggctggagatgtattgcgcacccctcaagcctgccaag
tcagct
```

B

```
ggtcctgaaactttatgtggtgctgaattagtagatgctttacaattcgta
tgtggtgatcgtggtttctatttcaacaaacctactggttacggttcttct
tctcgtcgtgctcctcaaactggtattgtagatgaatgttgtttccgttct
tgtgatttacgtcgtttagaaatgtactgtgctcctttaaaacctgctaaa
tctgct
```

C




42.

Figure 5: A) IGF-I native sequence coding for the mature protein. B) IGF-I optimized sequence according to chloroplast preferred codon usage. Note changes in red. C) IGF-I synthetic gene after recursive PCR.